Lu et al., http://www.jcb.org/cgi/content/full/jcb.201012063/DC1



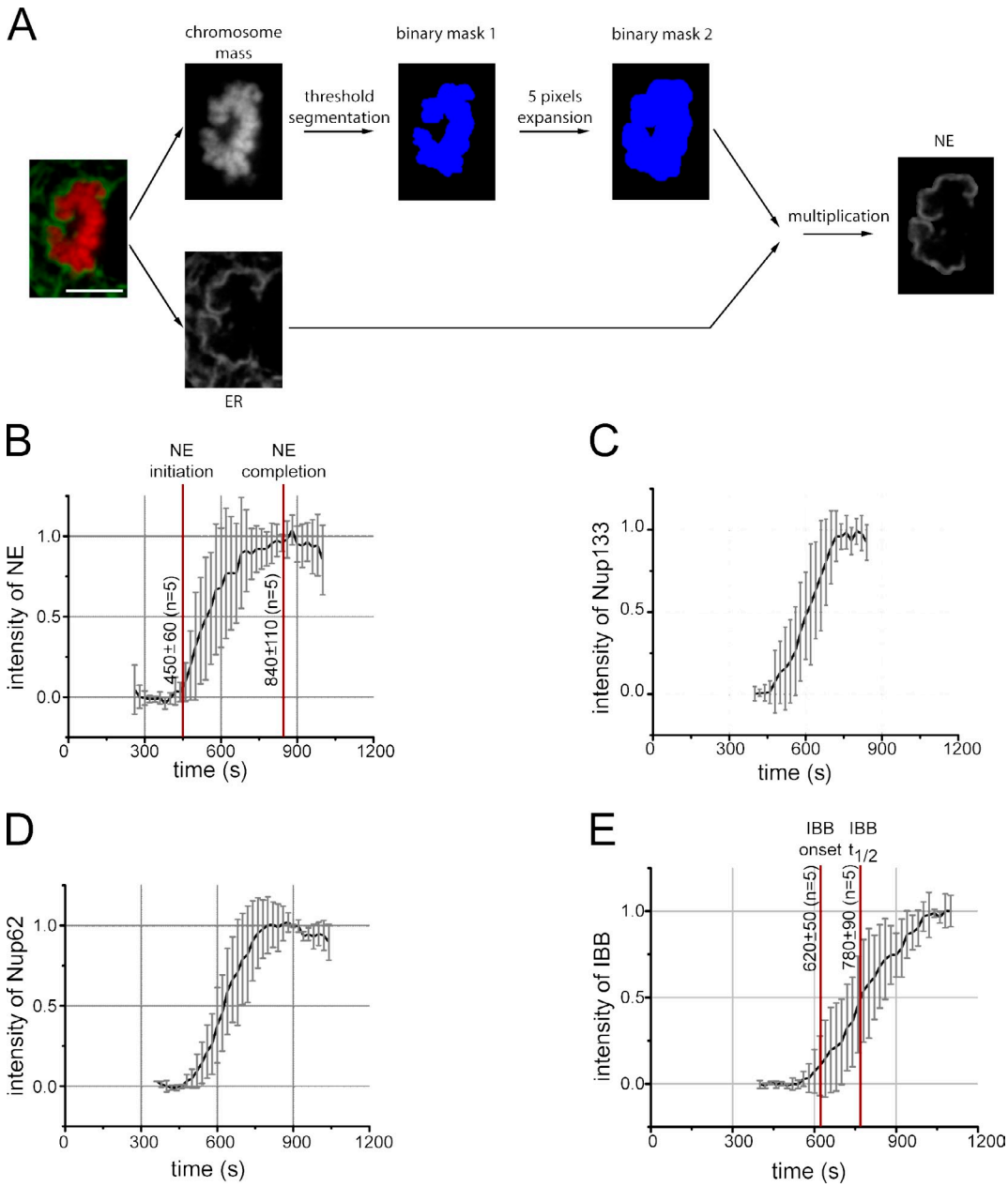



Figure S1. **Kinetics of nuclear envelope assembly, recruitment of Nup133 and Nup62, and import of IBB.** (A) Masking procedure to map the nuclear envelope for quantitative analysis. The example corresponds to a HeLa cell coexpressing GFP-Sec61β and H2B-mCherry. The region corresponding to the nuclear envelope (NE) was obtained by multiplying the image of the ER (GFP-Sec61β) with binary mask 2, which corresponds to the chromosome mass defined by the fluorescence signal of H2B (binary mask 1) expanded by 5 pixels. Bar, 5 µm. (B) Kinetics of nuclear envelope assembly expressed as fluorescence intensity ± SD ($n$ = 5). (C) Kinetics of Nup133 recruitment expressed as fluorescence intensity ± SD ($n$ = 5) from HeLa cells coexpressing GFP-Nup133 and H2B-mCherry. (D) Kinetics of Nup62 recruitment expressed as fluorescence intensity ± SD ($n$ = 4) from HeLa cells coexpressing GFP-Nup62 and H2B-mCherry. (E) Kinetics of IBB import expressed as fluorescence intensity ± SD ($n$ = 5) from HeLa cells coexpressing GFP-Sec61β and IBB-tomato. IBB $t_{1/2}$ is the mean time at which the imported IBB reached half maximal intensity.

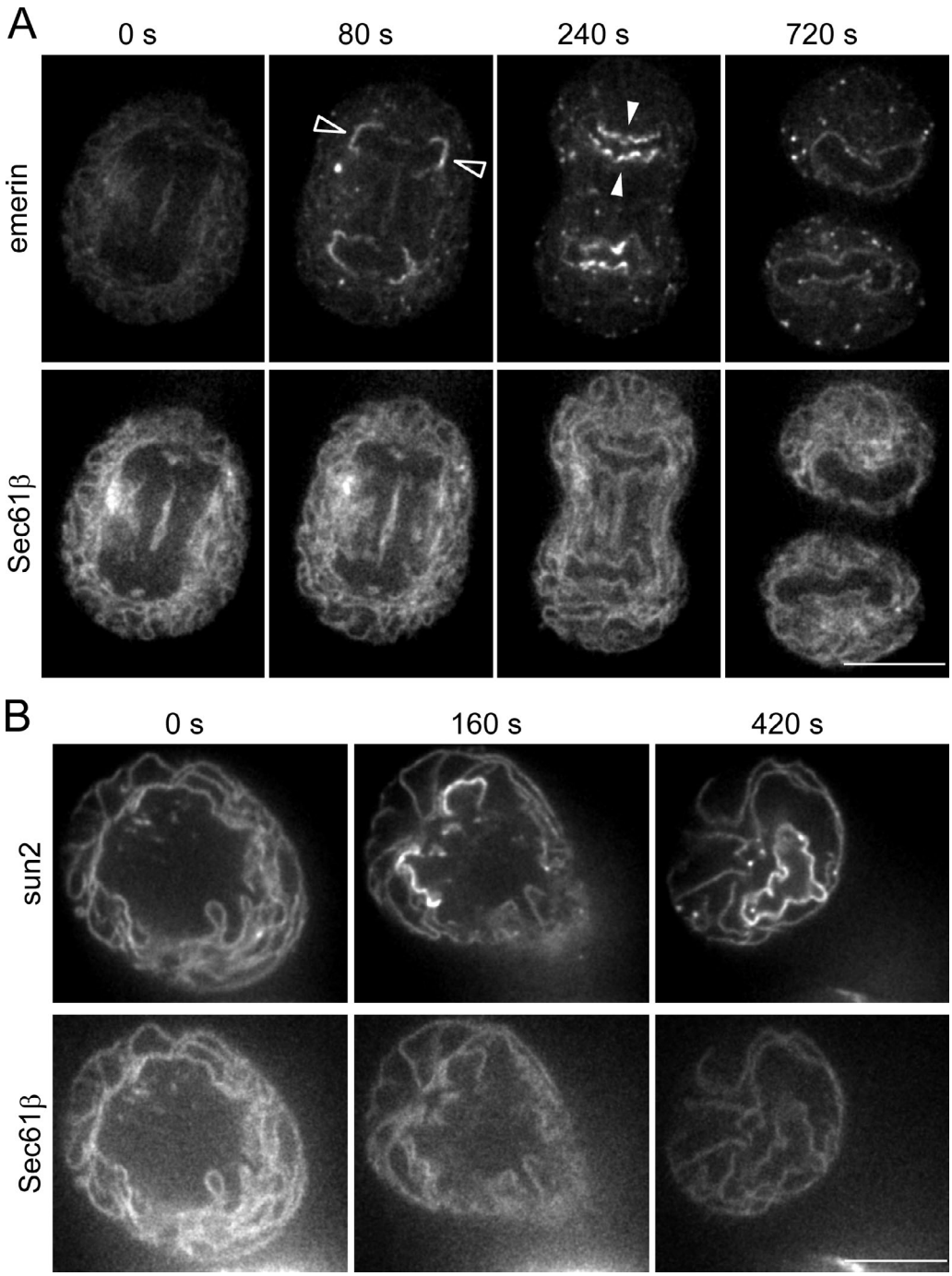



Figure S2. **Accumulation of emerin and sun2 on the nascent nuclear envelope.** (A and B) 2D time-lapse imaging of mitotic HeLa cells expressing mCherry-Sec61β and the nuclear envelope proteins emerin-GFP (A) or sun2-GFP (B). Emerin first localizes with Sec61β to the rim (80 s, open arrowheads) and then concentrates along the center of the chromosome mass (240 s, closed arrowheads) before becoming evenly distributed throughout the nuclear envelope (720 s). Bars, 10 µm.

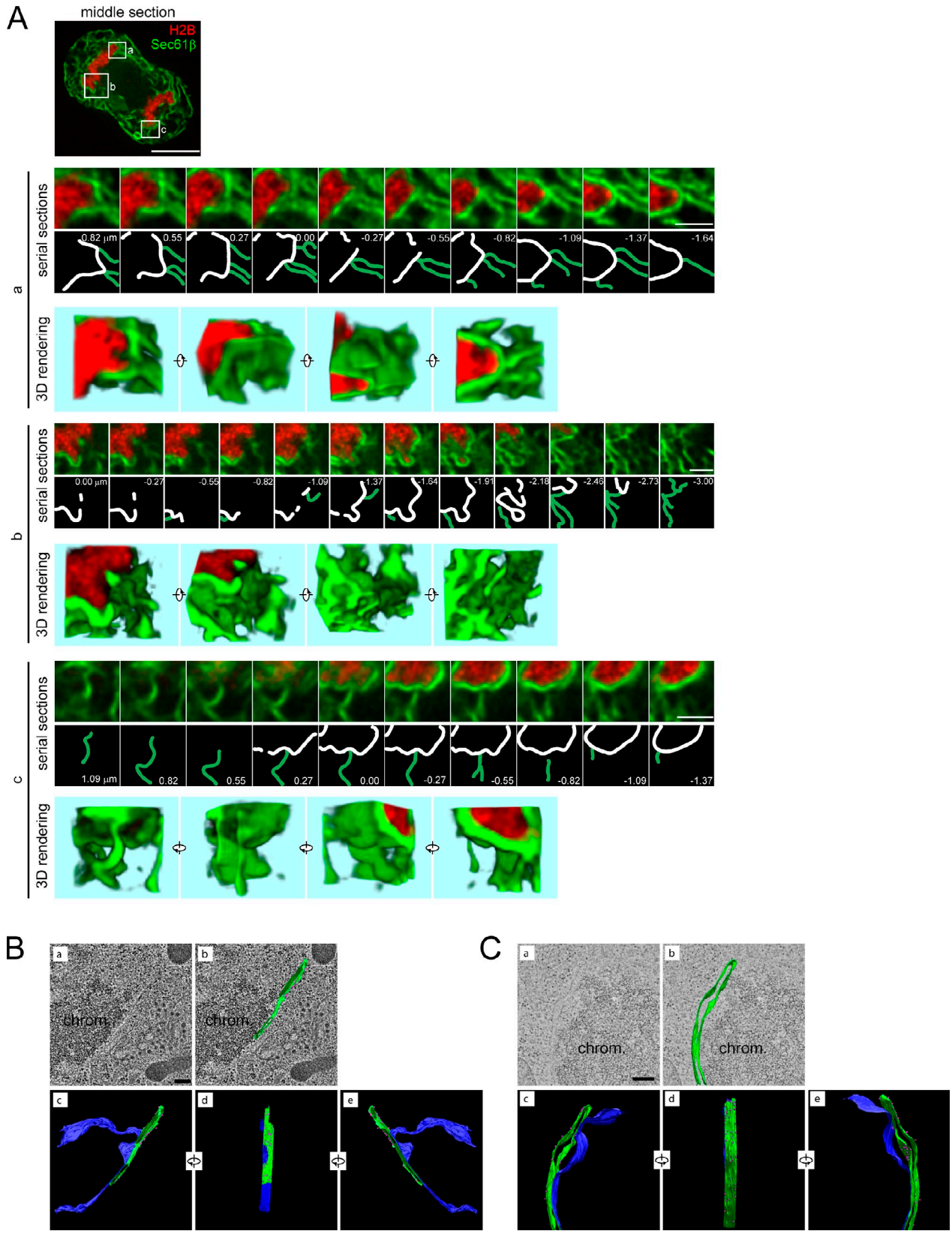



Figure S3. **Direct contribution of ER cisternae to the assembly of the nuclear envelope.** (A) The images in this figure derive from three different regions of the HeLa cell shown in Fig. 4 A. For a detailed description, see the legend of Fig. 4 A. ER, green lines; nuclear envelope, white lines. (B and C) EM tomograms of the nascent nuclear envelope from postmitotic IEC6 (B) and BSC1(C) cells imaged during anaphase. ER, ribosome, and the surface of the chromosome mass are colored in green, magenta, and blue, respectively. For a detailed description, see the legend of Fig. 4 C. Chrom., chromosome. Bars: (A, top) 10 µm; (A, a–c) 2 µm; (B and C) 200 nm.

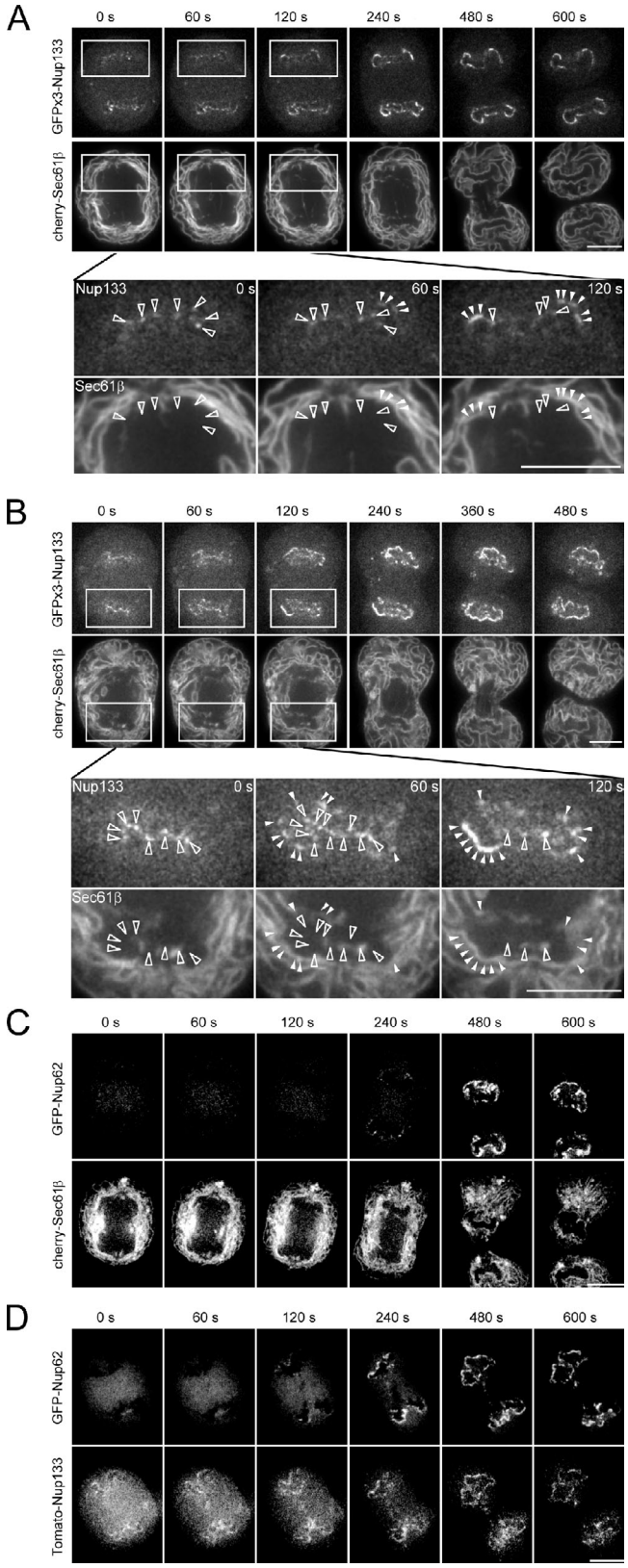



Figure S4. **Sequential assembly of Nup133 and Nup62 during mitosis occurs on the nascent nuclear envelope.** (A and B) Mitotic assembly of higher order Nup107–160 complex structures is restricted to sites on the nascent nuclear envelope. 2D time-lapse series from two different mitotic HeLa cells expressing GFPx3-Nup133 and mCherry-Sec61β showing the recruitment of Nup133 onto the nuclear envelope. For a detailed description, see the legend of Fig. 5 (C and D). Boxed regions are enlarged in the images below. Open arrowheads show Nup133 associated with kinetochores. Closed arrowheads show Nup133 recruited to nascent nuclear envelope. (C) Time-lapse series from a mitotic HeLa cell expressing GFP-Nup62 and mCherry-Sec61β showing that Nup62 is only recruited onto sites already containing nuclear envelope membrane. (D) Time-lapse series from a mitotic HeLa cell expressing GFP-Nup62 and tomato-Nup133 showing the sequential recruitment of Nup62 to sites already containing Nup133 on the outer boundary of the chromosome mass. Bars, 10 µm.

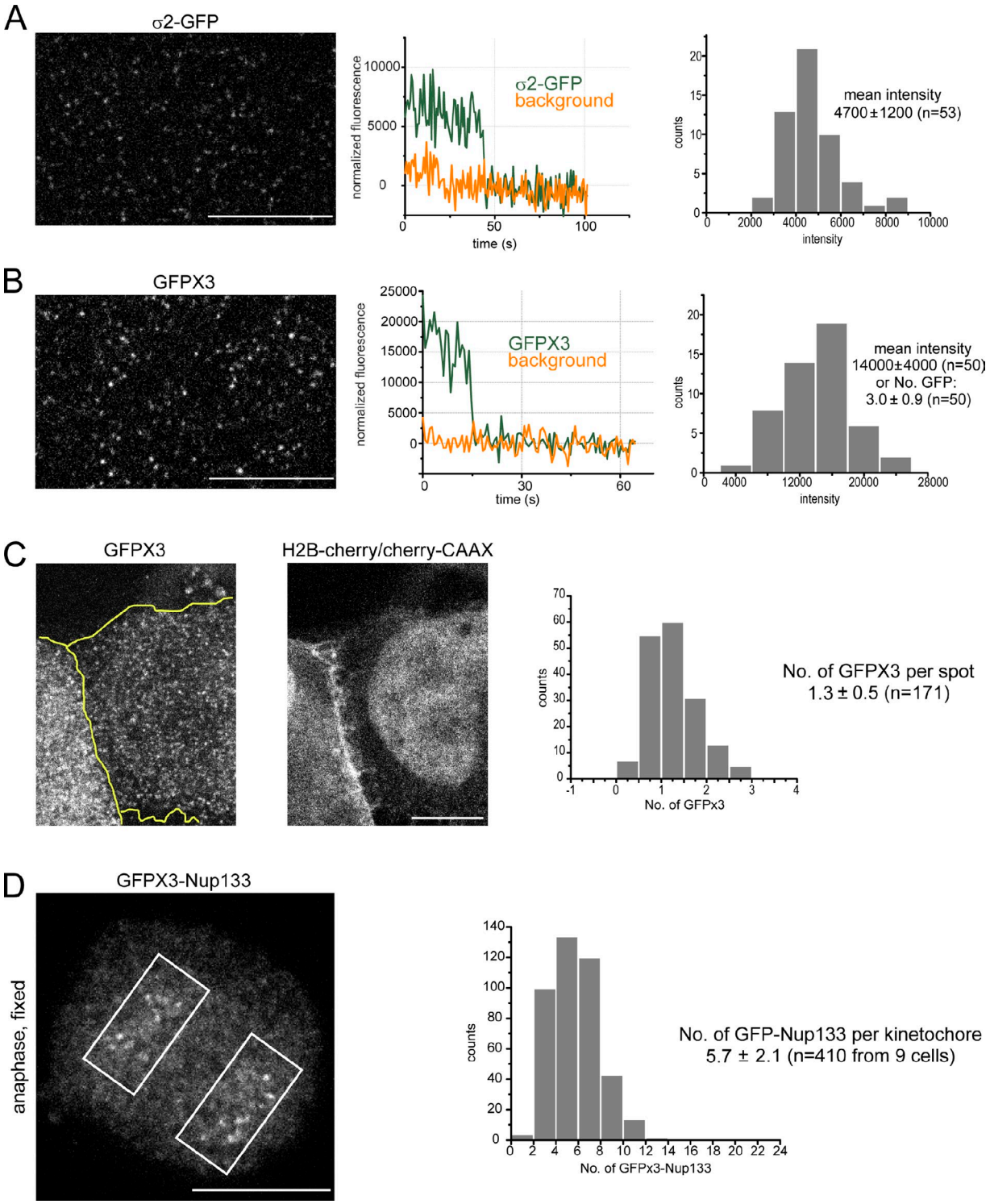
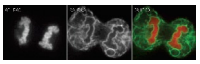
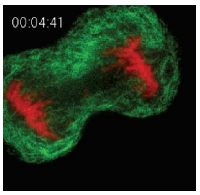


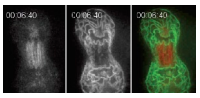

Figure S5. **Calibration of the fluorescence intensity of GFP and GFP×3.** (A and B) In vitro calibration. Lysates from cells expressing σ2-GFP (A) or GFP×3 (B) were plated onto the glass coverslip and imaged in 2D by time-lapse spinning-disk confocal microscopy until the fluorescence signal of the GFPs disappeared by photobleaching. Left images correspond to the first images of the time series. Middle graphs are typical single-molecule photobleaching traces of σ2-GFP and GFP×3. The fluorescence intensity distributions for single σ2-GFP and GFP×3 molecules are shown on the right. The fluorescence intensity of GFP×3 is approximately three times the intensity of single GFP. The data of A and B represent the results from three experiments. (C) In vivo validation. HeLa cells were simultaneously transfected with plasmids encoding GFP×3, H2B-mCherry, and mCherry-CAAX. (left) GFP×3 image. (middle) H2B-mCherry and mCherry-CAAX image. A cell expressing a very low level of GFP×3 (right cell in left and middle images) was selected for analysis. The yellow contours (left) outline the plasma membrane marked by the location of mCherry-CAAX. The fluorescence intensity distribution indicates that on average, each fluorescence spot corresponds to one GFP×3. (D) Content of GFP×3-Nup133 within kinetochores during anaphase. HeLa cells expressing GFP×3-Nup133 were fixed during anaphase, imaged in 3D, and subjected to fluorescence intensity quantification. The left image shows the middle section of a cell containing bright fluorescent spots of GFP×3-Nup133 located in kinetochores. (right) Fluorescence intensity distribution of the GFP×3-Nup133 signal on kinetochores. The boxes indicate kinetochores on the two daughter chromosome masses. The data of C and D represent the results from three and nine cells, respectively. Bars, 10 μm.



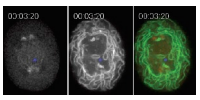
Video 1. **Dynamics of mitotic nuclear envelope assembly from ER cisternae.** A mitotic HeLa cell expressing GFP-Sec61β (green) and H2B-mCherry (red) was imaged every 20 s from the onset of anaphase at 0 s until completion of the nuclear envelope assembly. The 2D time-lapse video was acquired with a spinning-disk confocal microscope. The display rate is 10 frames per second, and the time stamp shows minutes and seconds.



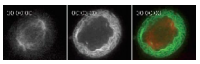

Video 2. **Dynamics of mitotic nuclear envelope assembly in a BSC1 cell.** A mitotic BSC1 cell expressing GFP-Sec61β (green) and H2B-mCherry (red) was imaged every 20 s during nuclear envelope reformation. Note the dense ER cisternae around the chromosome mass before the initiation of nuclear envelope assembly. The 2D time-lapse video was acquired with a spinning -disk confocal microscope. The display rate is 10 frames per seconds, and the time stamp shows minutes and seconds.



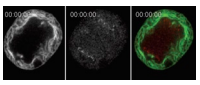
Video 3. **Dynamics of spindle microtubules and nuclear envelope assembly.** A mitotic HeLa cell expressing GFP-Sec61β (green) and mCherry-tubulin (red) was imaged every 20 s during nuclear envelope assembly. The 2D time-lapse video was acquired with a spinning-disk confocal microscope. The display rate is 10 frames per second, and the time stamp shows minutes and seconds.



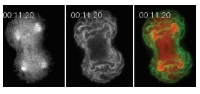
Video 4. **Effect of nocodazole on the dynamics of spindle microtubules and nuclear envelope assembly.** A mitotic HeLa cell expressing GFP-Sec61β (green) and mCherry-tubulin (red) was treated with 33 μM nocodazole at early anaphase (0 s) and imaged every 20 s during nuclear envelope assembly. Blue dots mark the site of nuclear envelope initiation at the pole distal side of the chromosome mass. The 2D time-lapse video was acquired with a spinning-disk confocal microscope. The display rate is 10 frames per second, and the time stamp shows minutes and seconds.



Video 5. **Effect of taxol on the dynamics of spindle microtubules and nuclear envelope assembly.** A mitotic HeLa cell expressing GFP-Sec61β (green) and mCherry-tubulin (red) was treated with 2 μM taxol at early anaphase (0 s) and imaged every 20 s during nuclear envelope assembly. Blue dots mark a gap in the nuclear envelope caused by exclusion by the stabilized spindle microtubules. The 2D time-lapse video was acquired with a spinning-disk confocal microscope. The display rate is 10 frames per second, and the time stamp is shown as minutes and seconds.



Video 6. **Dynamics of mitotic assembly of Nup133 relative to the nascent nuclear envelope.** A mitotic HeLa cell expressing mCherry-Sec61β (green) and GFPx3-Nup133 (red) was imaged every 10 s during nuclear envelope assembly. The 2D time-lapse video was acquired with a spinning-disk confocal microscope. The display rate is 10 frames per second, and the time stamp shows minutes and seconds.



Video 7. **Dynamics of IBB import during nuclear envelope assembly.** A mitotic HeLa cell expressing GFP-Sec61β (green) and IBB-tomato (red) was imaged every 20 s from anaphase onset (0 s) until completion of the nuclear envelope assembly. Note that IBB import begins before completion of the nuclear envelope. The 2D time-lapse video was acquired with a spinning-disk confocal microscope. The display rate is 10 frames per second, and the time stamp shows minutes and seconds.